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## **Domestic shower hose biofilms contain fungal species capable of causing opportunistic infection**

John Moat<sup>1\*</sup>, Athanasios Rizoulis<sup>2</sup>, Graeme Fox<sup>1</sup> & Mathew Upton<sup>1,3#</sup>

<sup>1</sup>Faculty of Medical and Human Sciences and <sup>2</sup>School of Earth, Atmospheric and Environmental Sciences, The University of Manchester, Manchester, M13 9WL, UK.

<sup>3</sup>Plymouth University Peninsula Schools of Medicine and Dentistry, Plymouth, PL4 8AA, UK.

#Author for correspondence

Dr Mathew Upton

School of Biomedical and Healthcare Sciences, Plymouth University Peninsula Schools of Medicine and Dentistry, Portland Square, Drake Circus, Plymouth, PL4 8AA

Tel: +44 1752 5884466

Email: [mathew.upton@plymouth.ac.uk](mailto:mathew.upton@plymouth.ac.uk)

\*Current Address – AV Hill Building, University of Manchester, Rumford Street, Manchester. M13 9PT

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## **Abstract**

The domestic environment can be a source of pathogenic bacteria. We show here that domestic shower hoses may harbour potentially pathogenic bacteria and fungi. Well-developed biofilms were physically removed from the internal surface of shower hoses collected in four locations in England and southern Scotland. Amplicon pyrosequencing of 16S and 18S rRNA targets revealed the presence of common aquatic and environmental bacteria, including members of the Actinobacteria, Alphaproteobacteria, Bacteroidetes and non-tuberculous Mycobacteria. These bacteria are associated with infections in immunocompromised hosts and are reported to be causes of water-acquired infection. In addition, this study represents the first detailed analysis of fungal populations in shower systems and revealed the presence of sequences related to *Exophiala mesophila*, *Fusarium fujikuroi* and *Malassezia restricta*. These organisms can be associated with the environment and healthy skin, but also with infection in compromised and immuno-competent hosts and occurrence of dandruff. Domestic showering may result in exposure to aerosols of bacteria and fungi that are potentially pathogenic and toxigenic. It may be prudent to ensure their eradication by the use of disinfectants, or replacement of hoses, where immuno-compromised persons are present.

## Introduction

Water supplies in domestic environments have been the focus of studies into transmission of opportunistic infections with environmental organisms, usually in people who have a compromised immune system. It has been suggested that environmental organisms, including *Legionella* can become established in shower hoses, subsequently being aerosolised and inhaled leading to infection (Pedro-Botet *et al.* 2002). A number of studies, using culture-based and culture-independent methods, have attempted to characterise the organisms present in these aerosols and the surrounding environment, demonstrating the presence of potentially pathogenic biofilm forming Gram-negative bacteria in hospital water systems (Decker and Palmore 2013). Culture-independent methods have been used to demonstrate the presence of potentially pathogenic bacteria on domestic shower curtains (Kelley *et al.* 2004) and shower-heads (Feazel *et al.* 2009). Moreover, recent molecular and culture-based work has demonstrated the presence of non-tuberculous Mycobacteria (NTM) in domestic water (Thomson *et al.* 2013) and demonstrated infection acquired from aerosols of this water (Falkinham *et al.* 2008; Thomson *et al.* 2013). O'Brien and colleagues (O'Brien *et al.* 2000) have suggested that our increased tendency to shower, rather than take a bath, has contributed to a rise in NTM disease.

In contrast to the significant body of knowledge surrounding the bacterial components of domestic water system biofilms, there is currently a relatively limited understanding of the fungal members of these communities and none of the previous studies of shower systems have included detailed analysis of fungal species.

We have used culture-independent methods to investigate the bacterial and fungal diversity of biofilms recovered from domestic shower hoses, revealing the presence of

several genera or species that represent a risk to immuno-compromised hosts.

## **Materials and Methods**

### **Shower hose samples**

A total of four hose samples were examined; one hose was recovered from a hotel in Northwest England (NW; latitude 53.414°N, longitude -2.124°W) and a further three from domestic settings in Southern Scotland (Fife; 56.135°N, -3.376°W) and in the Southeast of England (SE1; 51.167°N, 1.289°W & SE2; 51.424°N, 0.560°W). The hoses examined were removed from standard wall-mounted electric showers, so will have been supplied with pre-mixed warm water. The SE1 hose was recovered from a shower that had been installed less than a month previous to removal of the hose. Other hoses had been in place for an undetermined, but extended time. All samples were obtained with the full consent of the property owners, without restriction.

All wet hoses were filled with tap water at source, their ends being sealed with sterile screw caps, before being transported to Manchester at ambient temperature. On arrival, hoses were stored at 4°C and were analysed within 48 hrs.

### **Amplicon pyrosequencing for characterization of bacterial and fungal biofilm communities**

Pyrosequencing of rRNA amplicons was employed to allow characterisation of the bacteria and fungi forming established biofilms on shower hoses. Although limited in sample numbers, this work was carried out using hoses recovered from England and Scotland, to give an indication of diversity over a geographically dispersed area, supplied by different water sources, rather than to generate a comprehensive catalogue of microbial diversity in this environment.

Hoses were surface sterilised with ethanol and aseptically sectioned into 7cm lengths and the biofilm was physically removed using a sterile scraper and recovered into sterile Phosphate Buffered Saline (PBS). Biofilm material was recovered from sections of hose closest to each end of the hose. Samples were mechanically disrupted by vigorous mixing for 2 minutes in the presence of 2g of sterile coarse sand and 3mm diameter glass beads. Total DNA was recovered using the MoBio Soil extraction kit (Cambio Ltd, UK) and stored at -20°C. PCR of the V1-V2 hypervariable region of the bacterial 16S rRNA gene was performed using tagged fusion universal bacterial primers 27F (Lane, 1991) and 338R (Hamady *et al.* 2008), synthesised by IDTdna (Integrated DNA Technologies, Belgium). The fusion forward primer (5'

CCATCTCATCCCTGCGTGTCTCCGACTCAGNNNNNNNNNNNAGAGTTTGAT  
GMTGGCTCAG 3') contained the 454 Life Sciences "Lib-L Primer A", a 4 base "key" sequence (TCAG), a unique ten-base barcode "MID" sequence for each sample (N), and bacterial primer 27F (underlined). The reverse fusion primer (5' CCTATCCCCTGTGTGCCTTGGCAGTCTCAGTGCTGCCTCCCGTAGGAGT 3') contained the 454 Life Sciences "Lib-L Primer B", a 4 base "key" sequence (TCAG), and bacterial primer 338R (underlined). PCR of the fungal 18S rRNA small subunit was carried out using tagged forward fusion primer FU18S1 (5'-GGAAACTCACCAGGTCCAGA-3') and reverse fusion primer Nu-SSU-1536 (5'-ATTGCAATGCYCTATCCCCA-3') (Gangneux *et al.* 2011).

The PCR amplification was performed in 50 µl volume reactions using 0.5 µl (2.5 units) FastStart High Fidelity DNA polymerase (Roche Diagnostics GmbH, Mannheim, Germany), 1.8 mM MgCl<sub>2</sub>, 200 µM of each dNTP, 0.4 µM of each forward and reverse fusion primers. The PCR conditions included an initial

denaturing step at 95°C for 2 min, followed by 35 cycles of 95°C for 30 sec, 55°C for 30 sec, 72°C for 45 sec, and a final elongation step at 72°C for 5 min.

The 16S and 18S rRNA gene fragments were loaded onto an agarose gel and, following gel electrophoresis, bands of the correct fragment size (approximately 410 bp) were excised, purified using a QIAquick gel extraction kit (Qiagen, GmbH, Hilden, Germany) and eluted in 30 µl of DNase free H<sub>2</sub>O. The purified PCR products were quantified using a Life Technologies Qubit 2.0 fluorometer and the fragment size confirmed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). PCR products were normalised and pooled at equimolar concentrations. The emulsion PCR and the pyrosequencing runs were performed at the University of Manchester DNA Sequencing Facility, using a Roche 454 Life Sciences GS Junior system (454 Life Sciences, Branford, CT, USA).

The 454 pyrosequencing reads were analysed using Qiime release 1.8.0 (Caporaso *et al.* 2010), and de-noising and chimera removal was performed in Qiime during operational taxonomic unit (OTU) picking (at 97% sequence similarity) with usearch (Edgar, 2010). Taxonomic classification of the 16S rRNA bacterial reads was performed in Qiime using the uclust method (Edgar, 2010) against the Greengenes database, release 08/2013 (McDonald *et al.* 2012). Taxonomic classification of the 18S rRNA fungal reads was carried out in Qiime using uclust against the Silva release 111 database (Yilmaz *et al.* 2014). In addition, the closest GenBank match for the OTUs (a representative sequence for each OTU was used) that contained the highest number of reads was identified by Blastn nucleotide search (Altschul *et al.* 1990). OTU clustering was carried out on the log-transformed abundances of the identified OTUs, using the Bray Curtis similarity method. Cluster analysis was carried out using the PRIMER v6 software (PRIMER-E, Plymouth, UK) (Clarke, 1993).

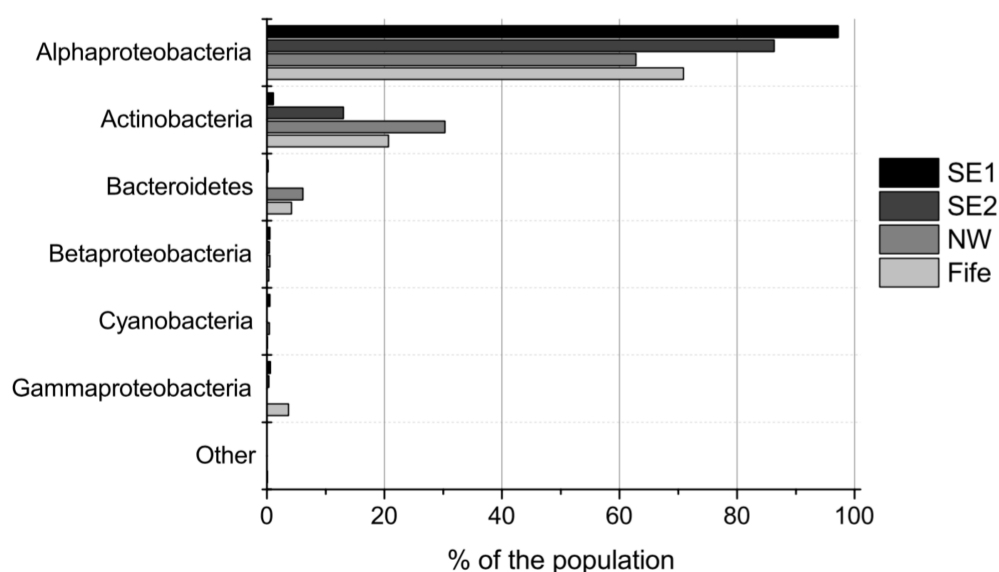
All raw sequence data relating to this work have been deposited at the Sequence Read Archive under BioProject number PRJNA267447.

## Results

Following bacterial 16S rRNA gene amplicon pyrosequencing, more than 20,000 reads were obtained from each sample (Table 1). Analysis of these reads indicated the presence of between 62 and 79 OTUs per sample, at the 97% sequence identity level (Table 1). Taxonomic classification showed that all bacterial communities were dominated by Alphaproteobacteria followed by Actinobacteria. Bacteroidetes related sequences were only present in the NW and Fife samples (Figure 1). All communities were dominated (47% to 80% of the total population) by sequences closely related (99-100% identity) to two Alphaproteobacterial species (Table 2), the rhizobial, N<sub>2</sub> fixing *Bradyrhizobium japonicum* SEMIA 5079 (Accession number, CP007569) and the photosynthetic *Blastomonas natatoria* DSM 3183 (NR\_040824). Sequences related to Alphaproteobacteria isolated from aqueous or soil environments, including *Novosphingobium* sp. AKB-2008-TA1 (AM989035), *Sphingopyxis* soli BL03 (NR\_116739), *Sphingomonas* soli T5-04 (NR\_041018), *Sphingomonas* sp. AKB-2008-TU3 (AM989063) and *Porphyrobacter donghaensis* SW-132 (NR\_025816) were also present in one or more samples (Table 2).

Samples NW and Fife were characterised by the presence of sequences closely related to two *Mycobacterium gordonae* strains [ATCC 14470 (NR\_118331) and DSM 44160 (NR\_114896)] isolated from gastric lavage (25.2% and 16.2% of all reads for each sample, respectively; Table 2). In contrast, 11.6% and 1% of the reads from the SE2 and SE1 samples, respectively, were more closely related to *M. mucogenicum* N248 (AY215289; Table 2).



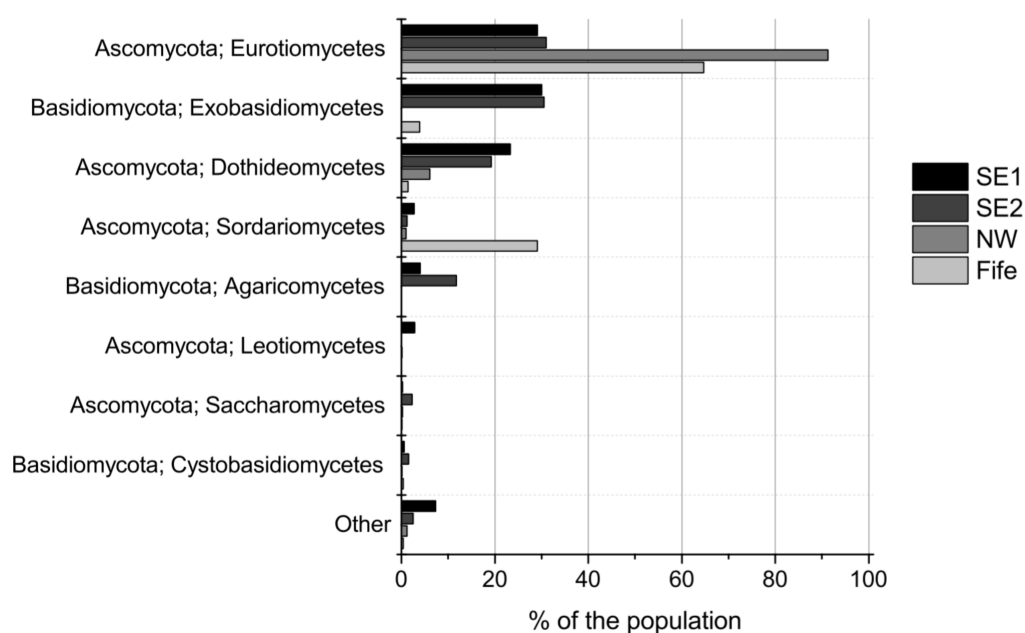


**Figure 1.** Bacterial taxonomic classification at the phylum level (class for the Proteobacteria). Only phyla/classes with more than 1% of the total number of reads are shown.

Bacteroidetes related sequences of the NW sample were mainly related to *Hydrothalea flava* CCUG 51397, recently described in Swedish water samples (Kämpfer *et al.* 2011), while in the Fife sample were affiliated to *Lacibacter cauensis* NBRC 104930 (Table 2), another recently described aquatic bacterium (Qu *et al.* 2009).

Fungal 18S rRNA gene amplicon pyrosequencing also generated more than 20,000 reads per sample (Table 1). The sequence data obtained revealed the presence of between 16 and 36 OTUs per sample, at the 97% sequence identity level (Table 3). The reads obtained were classified predominantly in the Ascomycota and Basidiomycota phyla, and mainly in the Eurotiomycetes, Exobasidiomycetes, Dothideomycetes, Sordariomycetes, Agaricomycetes class of fungi (Table 3; Figure 2). The majority of the fungal pyrosequencing reads from the NW and Fife samples

(89% and 64% respectively; Table 3) had 99% identity to *Exophiala mesophila* CBS 402.95 (JN856016), a waterborne species isolated from a shower joint (de Hoog *et al.* 2011). Only 20% of the reads in sample SE1 were affiliated to this species. In addition, 28.7% of the reads from the Fife sample had 100% identity to the rice pathogen *Fusarium fujikuroi* IMI 58289 (HF679024).

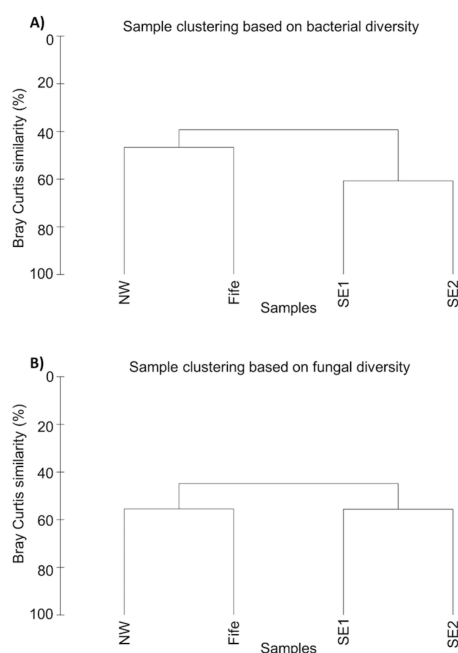


**Figure 2.** Fungal taxonomic diversity at the phylum; class level. Only phyla/classes with more than 0.5% of the total number of reads are shown.

The fungal populations of samples SE1 and SE2 displayed similar compositions (Table 3). Approximately 21% of the reads in these samples were closely related to *Malassezia restricta* CBS 7877 (EU192367), an organism sometimes associated with severe opportunistic infections (Arendrup *et al.* 2014), although they are also members of skin flora. Additional sequences (9%) were related to *Malassezia sympodialis* (EU192369).

Plant pathogen *Leptosphaeria maculans* JN3 (NW\_003533867) was represented by 17% of sequences. In sample SE2, 29% of the reads were affiliated to various *Penicillium* species (Table 3). Other fungal species detected in one or more of the samples of this study included airborne *Cryptococcus vishniacii*, *Aspergillus glaucus*, *Cladosporium uredinicola* CPC 5390, *Trametes sanguinea* and wood-rotting *Schizophyllum commune*, an emerging cause of sinusitis (Lorentz *et al.* 2012) (Table 3).

OTU clustering analysis (Figure 3) and taxonomic classification (Tables 2 and 3) revealed similarities in the bacterial and fungal communities in samples SE1 and SE2, whereas samples NW and Fife were more similar to each other than the two samples from the South East.



**Figure 3.** Sample clustering based on bacterial (A) or fungal (B) diversity. Clustering was carried out on the log transformed abundances of the identified OTUs, using the Bray Curtis similarity method.

## Discussion

The environment around us hosts a significant number of microbes and, in domestic settings, it is suggested that the majority are of human origin (Hospodsky *et al.* 2012). These bacteria and fungi persist in the environment for a variable length of time, which will affect the opportunity for acquisition by a susceptible host. Previous studies have highlighted the importance of waterborne *Legionella* species in domestic and healthcare settings (Fields *et al.* 2002; Leoni *et al.* 2005; Decker and Palmore 2013), but there is now growing interest in other bacteria that may infect immuno-compromised hosts. In the current study, we have used pyrosequencing approaches to characterise the bacterial and fungal inhabitants of domestic shower hoses from four sites in England and Scotland, demonstrating the presence of bacteria and fungi that may represent a threat to immuno-compromised hosts, and certain fungi that can be more overtly pathogenic.

Amplicon pyrosequencing revealed a maximum of 79 bacterial and 36 fungal OTUs per sample, with most of the sequences clustered within a small number of OTUs. These relatively low levels of diversity in the samples examined is similar to that reported for showerhead communities in the USA and probably results from the low nutrient environment that is present in the water supplying the hoses (Feazel *et al.* 2009). Cluster analysis and taxonomic classification of all the microbial communities revealed that two geographically distant samples (NW and Fife) shared some similarities and a number of bacterial and fungal species were present across all samples (in some cases in abundance). This may indicate that specific organisms preferentially colonise and establish within the biofilm communities that develop over time within domestic shower hoses. The ways in which these organisms interact and the dynamics of community succession warrant further investigation. The differences

in the bacterial and fungal communities observed in the samples could be attributed to a range of reasons, such as water composition in the different regions; water in Northwest England and Fife is soft, whereas it is generally medium-hard to hard in Southeast England (waterwise.org.uk). A larger and more detailed analysis of the impact of different physicochemical parameters of water on shower biofilm populations is warranted, though the similarities seen between the samples examined here suggest that some genera are widely dispersed, irrespective of water characteristics.

Environmental Alphaproteobacteria, Actinobacteria and Bacteroidetes dominated the bacterial communities. Several examples of the *Sphingomonadaceae* were observed (e.g. *Blastomonas*, *Novosphingobium*, *Sphingopyxis*, *Sphingomonas* and *Porphyrobacter*). Although only very rarely associated with infections, some of these species form extensive biofilms and it is suggested that waterborne members of the *Sphingomonadaceae* from hospital and domestic environments may be a reservoir for antibiotic resistance genes (Narciso-da-Rocha *et al.* 2014). *Porphyrobacter donghaensis* has been associated with environmental and hospital water in previous studies (Yoon *et al.* 2004; Furuhashi *et al.* 2007), but not with infection.

Samples contained sequences related to *Mycobacterium gordonae* and *M. mucogenicum*. *M. gordonae* has been reported in showerhead populations (Feazel *et al.* 2009). Members of the NTM are ubiquitous in the environment and have recently been associated with shower aerosol related infection in Australia (Thomson *et al.* 2013), in a study that reported links between strains of NTM from patients and their domestic environments. In a different study of showerheads and hose samples, Rhodes and colleagues (Rhodes *et al.* 2014) demonstrated the widespread occurrence of *Mycobacterium avium* subspecies *paratuberculosis* (Map), which is significantly

associated with development of Crohn's disease. Their findings supported the suggestion that shower aerosols may have an under-recognized role in the acquisition of Crohn's disease.

Of note in the current study was the lack of any sequence related to *Legionella* species, though previous studies have indicated low level occurrence (0.1% of total sequences) of these organisms in shower systems, using both 16S rRNA gene sequencing and quantitative PCR based methods (Feazel *et al.* 2009).

Fungal sequence reads were related to the Eurotiomycetes, Exobasidiomycetes, Dothideomycetes, Sordariomycetes, Agaricomycetes. In some cases, there was a predominance of *Exophiala mesophila*, an organism associated with cutaneous and subcutaneous infections (Zeng *et al.* 2007), was first isolated from silicone seals in a hospital shower room (Listemann and Freiesleben 1996). *Exophiala dermatitidis* has been recovered from drinking water in Slovenia, where the occurrence of these organisms have been studied in detail (Novak Babič *et al.* 2015). *Exophiala* species have been observed in high numbers in sauna facilities (Matos *et al.* 2002), domestic dishwashers (Gümral *et al.* 2015) and washing machines (Novak Babič *et al.* 2015). Although they rarely cause infections, these can be difficult to treat (Rimawi *et al.* 2013).

Also observed were sequences related to *Fusarium fujikuroi*. Members of the *Fusarium fujikuroi* species complex are predominantly plant pathogens, but they are being increasingly recognized as aetiological agents of infection in immunocompromised and immunocompetent hosts (Kebabci *et al.* 2014; Tortorano *et al.* 2014) and infection with some *Fusarium* species has been linked to colonization of hospital water systems (Anaissie *et al.* 2001). An additional potential risk with these

organisms is the generation of trichothecenes, protein synthesis inhibitors that are documented to cause intoxications in humans following consumption of food products colonised with *Fusarium* species (Murphy *et al.* 2006) and may lead to an increase in respiratory tract infections (Bhat *et al.* 1989). Given the toxicity of trichothecenes, concentrations in water emerging from showers should be investigated.

In samples from the Southeast of England, over 20% of the reads were related to *Malassezia restricta*, an organism associated with severe opportunistic infections (Arendrup *et al.* 2014), although they are also members of skin flora. Given recent evidence for an association between *Malassezia restricta* and dandruff (Gemmer *et al.* 2002; Clavaud *et al.* 2013), the implications of significant numbers of these organisms in the shower hose biofilms is clear. Overall, members of the *Malassezia* group are among the most common causes of superficial mycoses (Crespo-Erchiga and Florencio 2006) and dermatoses (Jagielski *et al.* 2014).

The UK Department of Health suggests that flexible hoses should not be used in high-risk situations (Best Practice Guidance; HTM 04-01 - Addendum: *Pseudomonas aeruginosa* – Advice for Augmented Care Units, March 2013). Such guidelines are implemented to reduce infection with *Pseudomonas aeruginosa*, but the data presented here suggests that effective management of biofilms in flexible hoses may control several additional opportunistic bacterial and fungal pathogens.

## Conclusions

Previous studies have catalogued the bacterial populations associated with showerheads and shower curtains, and our data corroborate these findings. Ours is the first study to describe in detail the fungal community members, some of which give

cause for concern. Although our findings from a small collection of samples are preliminary, more detailed investigations are justified. The role of different shower water supplies (e.g. tank vs mains feed) and shower hose materials should be established. The impact of frequency of shower use on biofilm populations and the rate of biofilm accumulation should be investigated. We support the suggestion in previous studies that potential pathogens residing in shower hose biofilms will periodically slough off the inner surface, being released and distributed in shower aerosols (Falkinham *et al.* 2008; Rhodes *et al.* 2014). It may be prudent to routinely decolonise or replace shower hoses in domestic and health-care settings, where immuno-compromised individuals are present.

### **Funding Information**

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**Acknowledgements:** All DNA sequence analysis was carried out at the University of Manchester DNA Sequencing Facility.



### **Table and Figure Legends**

**Table 1.** Number of bacterial 16S rRNA and fungal 18S rRNA pyrosequencing reads obtained in this study, reads remaining after denoising and chimera check, and observed OTUs at 97% ID similarity level.

**Table 2.** The closest phylogenetic relative of the identified bacterial OTUs of this study with the highest number of reads.

**Table 3.** The closest phylogenetic relative of the identified fungal OTUs of this study with the highest number of reads.

**Figure 1.** Bacterial taxonomic classification at the phylum level (class for the Proteobacteria). Only phyla/classes with more than 1% of the total number of reads are shown.

**Figure 2.** Fungal taxonomic diversity at the phylum; class level. Only phyla/classes with more than 0.5% of the total number of reads are shown.

**Figure 3.** Sample clustering based on bacterial (A) or fungal (B) diversity. Clustering was carried out on the log transformed abundances of the identified OTUs, using the Bray Curtis similarity method.

**Table 1.** Number of bacterial 16S rRNA and fungal 18S rRNA pyrosequencing reads obtained in this study, reads remaining after denoising and chimera check, and observed OTUs at 97% ID similarity level.

<b>Sample</b>	<b>Number of reads</b>	<b>Reads after denoising &amp; chimera check</b>	<b>Observed OTUs</b>	<b>Shannon</b>	<b>Chao1</b>
NW, bacterial	126502	121000	76	2.96	81.25
Fife, bacterial	33977	32366	62	3.08	62.91
SE1, bacterial	30496	29301	64	2.09	73.00
SE2, bacterial	25865	24933	79	3.23	84.14
<b>Total bacterial</b>	<b>216840</b>	<b>207600</b>	<b>129</b>	NA	NA
NW, fungal	42381	39311	27	0.73	27.5
Fife, fungal	31790	30182	16	1.36	16
SE1, fungal	33973	31366	36	3.50	36
SE2, fungal	21276	18362	28	3.12	31
<b>Total fungal</b>	<b>129420</b>	<b>119221</b>	<b>49</b>	NA	NA

**Table 2.** The closest phylogenetic relative of the identified bacterial OTUs of this study with the highest number of reads.

NW %	Fife %	SE1 %	SE2 %	Closest relative (accession number; phylum/class)	ID %	Environment/reference
0.1	27.9	58.6	20.0	<i>Blastomonas natatoria</i> DSM 3183 NR_040824; <i>Alphaproteobacteria</i>	99	Photosynthetic (Hiraishi et al. 2000)
25.2	18.9	21.2	28.7	<i>Bradyrhizobium japonicum</i> SEMIA 5079 CP007569; <i>Alphaproteobacteria</i>	99	Rhizobial, N <sub>2</sub> fixing (Siqueira et al. 2014)
32.1	0.2	0.8	4.1	<i>Bradyrhizobium japonicum</i> SEMIA 5079 CP007569; <i>Alphaproteobacteria</i>	100	Rhizobial, N <sub>2</sub> fixing (Siqueira et al. 2014)
2.0	0	8.7	10.1	<i>Novosphingobium</i> sp. AKB-2008-TA1 AM989035; <i>Alphaproteobacteria</i>	97	Lake water (Berg et al. 2009)
0	19.9	0	0	<i>Sphingopyxis soli</i> BL03 NR_116739; <i>Alphaproteobacteria</i>	99	Landfill soil (Choi et al. 2010)
0	0	0.0	7.4	<i>Sphingomonas soli</i> T5-04 NR_041018; <i>Alphaproteobacteria</i>	99	Soil (Yang et al. 2006)
0.4	0	1.0	6.0	<i>Sphingomonas</i> sp. AKB-2008-TU3 AM989063; <i>Alphaproteobacteria</i>	100	Lake water (Berg et al. 2009)
0	0	0	4.3	<i>Porphyrobacter donghaensis</i> SW-132 NR_025816; <i>Alphaproteobacteria</i>	100	Sea water (Yoon et al. 2004)
9.8	12.1	0	0.7	<i>Mycobacterium gordonae</i> DSM 44160 NR_114896; <i>Actinobacteria</i>	99	Gastric lavage (Bojalil et al. 1962; Lefmann et al. 2004)
13.5	0.2	0	0	<i>Mycobacterium gordonae</i> ATCC 14470 NR_118331; <i>Actinobacteria</i>	100	Gastric lavage (Bojalil et al. 1962)
1.9	3.9	0	0.1	<i>Mycobacterium gordonae</i> ATCC 14470 NR_118331; <i>Actinobacteria</i>	99	Gastric lavage (Bojalil et al. 1962)
0.1	0.0	1.0	11.6	<i>Mycobacterium mucogenicum</i> N248 AY215289; <i>Actinobacteria</i>	99	Clinical isolate (Hall et al. 2003)
5.4	0	0	0	<i>Hydrotalea flava</i> CCUG 51397 NR_117026; <i>Bacteroidetes</i>	98	Water (Kämpfer et al. 2011)
0.1	4.2	0	0	<i>Lacibacter cauensis</i> NBRC 104930 NR_114273; <i>Bacteroidetes</i>	93	Lake sediment (Qu et al. 2009)
9.4	12.6	8.7	6.9	Other		

**Table 3.** The closest phylogenetic relative of the identified fungal OTUs of this study with the highest number of reads.

NW %	Fife %	SE1 %	SE2 %	Closest relative (accession number; fungal class)	ID %	Environment/reference
89.3	64.1	19.9	0.4	<i>Exophiala mesophila</i> CBS 402.95 JN856016; <i>Eurotiomycetes</i>	99	Shower joint, waterborne (de Hoog et al. 2011)
0	3.8	20.5	20.9	<i>Malassezia restricta</i> CBS 7877 EU192367; <i>Exobasidiomycetes</i>	99	Healthy human skin
0	0	9.1	8.9	<i>Malassezia sympodialis</i> EU192369; <i>Exobasidiomycetes</i>	100	Healthy human skin
5.9	1.4	17.8	16.6	<i>Leptosphaeria maculans</i> JN3 NW_003533867; <i>Dothideomycetes</i>	99	Plant pathogen (Rouxel et al. 2011)
0	0	4.2	28.8	(various <i>Penicillium</i> species), e.g. <i>Penicillium solitum</i> 20-01 JN642222; <i>Eurotiomycetes</i>	100	Soil (Eldarov et al. 2012)
1.0	28.7	1.9	0.5	<i>Fusarium fujikuroi</i> IMI 58289 HF679024; <i>Sordariomycetes</i>	100	Rice pathogen (Wiemann et al. 2013)
1.1	0	4.5	1.7	<i>Cryptococcus vishniacii</i> AB032657; <i>Tremellomycetes</i>	100	Unknown (Takashima and Nakase 1999)
0	0	3.8	1.0	<i>Aspergillus glaucus</i> AF548072; <i>Eurotiomycetes</i>	100	Airborne (Wu et al. 2003)
0.2	0	4.2	0	<i>Cladosporium uredinicola</i> CPC 5390 AY251097; <i>Dothideomycetes</i>	99	Fungal parasite (Braun et al. 2003)
0	0	0	4.1	<i>Trametes sanguinea</i> AB084608; <i>Agaricomycetes</i>	100	Tree root (Suhara et al. 2002)
0	0	0	4.0	<i>Schizophyllum commune</i> X54865; <i>Agaricomycetes</i>	100	Wood-rotting (Bruns et al. 1992)
2.5	1.9	14.1	13.1	Other		

## References

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990 Basic Local Alignment Search Tool. *Journal of Molecular Biology* **215**, 403-410.
- Anaissie, E. J., Kuchar, R. T., Rex, J. H., Francesconi, A., Kasai, M., Muller, F. M., Lozano-Chiu, M., Summerbell, R. C., Dignani, M. C., Chanock, S. J., and Walsh, T. J. 2001 Fusariosis associated with pathogenic fusarium species colonization of a hospital water system: a new paradigm for the epidemiology of opportunistic mold infections. *Clinical Infectious Diseases* **33**, 1871-1878.
- Arendrup, M. C., Boekhout, T., Akova, M., Meis, J. F., A., C. O., Lortholary, O., ESCMID and ECMM Mycology Group. 2014 ESCMID and ECMM joint clinical guidelines for the diagnosis and management of rare invasive yeast infections. *Clinical Microbiology and Infection Suppl 3*, 76-98.
- Berg, K. A., Lyra, C., Sivonen, K., Paulin, L., Suomalainen, S., Tuomi, P., and Rapala, J. 2009 High diversity of cultivable heterotrophic bacteria in association with cyanobacterial water blooms. *ISME Journal* **3**, 314-325.
- Bhat, R. V., Beedu, S. R., Ramakrishna, Y., and L., M. K. 1989 Outbreak of trichothecene mycotoxicosis associated with consumption of mould-damaged wheat production in Kashmir Valley, India. *Lancet* **7**, 35-37.
- Bojalil, L. F., Cerbón, J., and Trujillo, A. 1962 Adansonian classification of mycobacteria. *Journal of General Microbiology* **28**, 333-&.
- Braun, U., Crous, P. W., Dugan, F., Groenewald, J. Z., Sybren, G., and Hoog, D. 2003 Phylogeny and taxonomy of *Cladosporium*-like hyphomycetes, including *Davidiella* gen. nov., the teleomorph of *Cladosporium* s. str. *Mycological Progress* **2**, 3-18.
- Bruns, T. D., Vilgalys, R., Barns, S. M., Gonzalez, D., Hibbett, D. S., Lane, D. J., Simon, L., Stickel, S., Szaro, T. M., Weisburg, W. G., and Sogin, M. L. 1992 Evolutionary relationships within the Fungi: Analyses of nuclear small subunit rRNA sequences. *Molecular Phylogenetics and Evolution* **1**, 231-241.
- Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., Fierer, N., Peña, A. G., Goodrich, J. K., Gordon, J. I., Huttley, G. A., Kelley, S. T., Knights, D., Koenig, J. E., Ley, R. E., Lozupone, C. A., McDonald, D., Muegge, B. D., Pirrung, M., Reeder, J., Sevinsky, J. R., Tumbaugh, P. J., Walters, W. A., Widmann, J., Yatsunenko, T., Zaneveld, J., and Knight, R. 2010 QIIME allows analysis of high-throughput community sequencing data. *Nature Methods* **7**, 335-336.
- Choi, J.-H., Kim, M.-S., Jung, M.-J., Roh, S. W., Shin, K.-S., and Bae, J.-W. 2010 *Sphingopyxis soli* sp. nov., isolated from landfill soil. *International Journal of Systematic and Evolutionary Microbiology* **60**, 1682-1686.
- Clarke, K. R. 1993 Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18**, 117-143.
- Clavaud, C., Jourdain, R., Bar-Hen, A., Tichit, M., Bouchier, C., Pouradier, F., El Rawadi, C., Guillot, J., Menard-Szczebara, F., Breton, L., Latge, J. P., and Mouyna, I. 2013 Dandruff is associated with disequilibrium in the proportion of the major bacterial and fungal populations colonizing the scalp. *PLoS One* **8**, e58203.
- Crespo-Erchiga, V., and Florencio, V. D. 2006 Malassezia yeasts and pityriasis versicolor. *Current Opinion on Infectious Diseases* **19**, 139-147.

- de Hoog, G. S., Vicente, V. A., Najafzadeh, M. J., Harrak, M. J., Badali, H., and Seyedmousavi, S. 2011 Waterborne *Exophiala* species causing disease in cold-blooded animals. *Persoonia* **27**, 46-72.
- Decker, B. K., and Palmore, T. N. 2013 The role of water in healthcare-associated infections. *Current Opinion in Infectious Diseases* **26**, 345-51.
- Edgar, R. C. 2010 Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **26**, 2460-2461.
- Eldarov, M. A., Mardanov, A. V., Beletsky, A. V., Dzhavakhiya, V. V., Ravin, N. V., and Skryabin, K. G. 2012 Complete mitochondrial genome of compactin-producing fungus *Penicillium solitum* and comparative analysis of *Trichocomaceae* mitochondrial genomes. *FEMS Microbiology Letters* **329**, 9-17.
- Falkinham, J. O., 3rd, Iseman, M. D., de Haas, P., and van Soolingen, D. 2008 *Mycobacterium avium* in a shower linked to pulmonary disease. *Journal of Water Health* **6**, 209-13.
- Feazel, L. M., Baumgartner, L. K., Peterson, K. L., Frank, D. N., Harris, J. K., and Pace, N. R. 2009 Opportunistic pathogens enriched in showerhead biofilms. *Proceedings of the National Academy of Sciences, USA* **106**, 16393-16399.
- Fields, B. S., Benson, R. F., and Besser, R. E. 2002 Legionella and Legionnaires' disease: 25 years of investigation. *Clinical Microbiology Reviews* **15**, 506-26.
- Furuhata, K., Kato, Y., Goto, K., Saitou, K., Sugiyama, J., Hara, M., and Fukuyama, M. 2007 Identification of yellow-pigmented bacteria isolated from hospital tap water in Japan and their chlorine resistance. *Biocontrol Science* **12**, 39-46.
- Gangneux, C., Akpa-Vinceslas, M., Sauvage, H., Desaire, S., Houot, S., and Laval, K. 2011 Fungal, bacterial and plant dsDNA contributions to soil total DNA extracted from silty soils under different farming practices: Relationships with chloroform-labile carbon. *Soil Biology & Biochemistry* **43**, 431-437.
- Gemmer, C. M., DeAngelis, Y. M., Theelen, B., Boekhout, T., and Dawson, T. L. J. 2002 Fast, noninvasive method for molecular detection and differentiation of *Malassezia* yeast species on human skin and application of the method to dandruff microbiology. *Journal of Clinical Microbiology* **40**, 3350-3357.
- Gümrall, R., Özhak-Baysan, B., Tümgör, A., Saraçlı, M. A., Yıldırım, S. T., Ilkit, M., Zupančič, J., Novak-Babič, M., Gunde-Cimerman, N., Zalar, P., and de Hoog, G. S. 2015 Dishwashers provide a selective extreme environment for human-opportunistic yeast-like fungi. *Fungal Biology* **115**, 997-1007.
- Hall, L., Doerr, K. A., Wohlfel, S. L., and Roberts, G. D. 2003 Evaluation of the MicroSeq system for identification of mycobacteria by 16S ribosomal DNA sequencing and its integration into a routine clinical mycobacteriology laboratory. *Journal of Clinical Microbiology* **41**, 1447-1453.
- Hamady, M., Walker, J. J., Harris, J. K., Gold, N. J., and Knight, R. 2008 Error-correcting barcoded primers for pyrosequencing hundreds of samples in multiplex. *Nature Methods* **5**, 235-237.
- Hiraishi, A., Kuraishi, H., and Kawahara, K. 2000 Emendation of the description of *Blastomonas natatoria* (Sly 1985) Sly and Cahill 1997 as an aerobic photosynthetic bacterium and reclassification of *Erythromonas ursincola* Yurkov et al. 1997 as *Blastomonas ursincola* comb. nov. *International Journal of Systematic and Evolutionary Microbiology* **50**, 1113-1118.

- Hospodsky, D., Qian, J., Nazaroff, W. W., Yamamoto, N., Bibby, K., Rismani-Yazdi, H., and Peccia, J. 2012 Human occupancy as a source of indoor airborne bacteria. *PLoS One*, e34867.
- Jagielski, T., Rup, E., Ziolkowska, A., Roeske, K., Macura, A. B., and Bielecki, J. 2014 Distribution of *Malassezia* species on the skin of patients with atopic dermatitis, psoriasis, and healthy volunteers assessed by conventional and molecular identification methods. *BMC Dermatology* **14**, 3.
- Kämpfer, P., Lodders, N., and Falsen, E. 2011 *Hydrotalea flava* gen. nov., sp. nov., a new member of the phylum *Bacteroidetes* and allocation of the genera *Chitinophaga*, *Sediminibacterium*, *Lacibacter*, *Flaviumibacter*, *Flavisolibacter*, *Niabella*, *Niastella*, *Segetibacter*, *Parasegetibacter*, *Terrimonas*, *Ferruginibacter*, *Filimonas* and *Hydrotalea* to the family *Chitinophagaceae* fam. nov. *International Journal of Systematic and Evolutionary Microbiology* **61**, 518-523.
- Kebabci, N., van Diepeningen, A. D., Ener, B., Ersal, T., Meijer, M., Al-Hatmi, A. M., Ozkocaman, V., Ursavas, A., Cetinoglu, E. D., and Akalin, H. 2014 Fatal breakthrough infection with *Fusarium andiyazi*: new multi-resistant aetiological agent cross-reacting with *Aspergillus galactomannan* enzyme immunoassay. *Mycoses* **57**, 249-255.
- Kelley, S. T., Theisen, U., Angenent, L. T., St. Amand, A., and Pace, N. R. 2004 Molecular Analysis of Shower Curtain Biofilm Microbes. *Applied and Environmental Microbiology* **70**, 4187-4192.
- Lane, D. J. 1991 16S/23S rRNA sequencing. In "Nucleic Acid Techniques in Bacterial Systematics" (E. Stackebrandt and M. Goodfellow, eds.), pp. 115–175. John Wiley and Sons, New York, NY.
- Lefmann, M., Honisch, C., Böcker, S., Storm, N., von Wintzingerode, F., Schlötelburg, C., Moter, A., van den Boom, D., and Göbel, U. B. 2004 Novel mass spectrometry-based tool for genotypic identification of mycobacteria. *Journal of Clinical Microbiology* **42**, 339-346.
- Leoni, E., De Luca, G., Legnani, P. P., Sacchetti, R., Stampi, S., and Zanetti, F. 2005 *Legionella* waterline colonization: detection of *Legionella* species in domestic, hotel and hospital hot water systems. *Journal of Applied Microbiology* **98**, 373-379.
- Listemann, H., and Freiesleben, H. 1996 *Exophiala mesophila* spec. nov. *Mycoses* **39**, 1-3.
- Lorentz, C., Rivier, A., Debourgogne, A., Sokolowska-Gillois, J., Vignaud, J. M., Jankowski, R., and Machouart, M. 2012 Ethmoido-maxillary sinusitis caused by the basidiomycetous fungus *Schizophyllum commune*. *Mycoses* **55**, e8-12.
- Matos, T., de Hoog, G. S., de Boer, A. G., de Crom, I., and Haase, G. 2002 High prevalence of the neurotrope *Exophiala dermatitidis* and related oligotrophic black yeasts in sauna facilities. *Mycoses* **45**, 373-377.
- McDonald, D., Price, M. N., Goodrich, J., Nawrocki, E. P., DeSantis, T. Z., Probst, A., Andersen, G. L., Knight, R., and Hugenholtz, P. 2012 An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME Journal* **6**, 610-618.
- Murphy, P. A., Hendrich, S., Landgren, C., and Bryant, C. M. 2006 Food Mycotoxins: An Update. *Journal of Food Science* **71**, 51–65.
- Narciso-da-Rocha, C., Vaz-Moreira, I., and Manaia, C. M. 2014 Genotypic diversity and antibiotic resistance in *Sphingomonadaceae* isolated from hospital tap water. *Science of the Total Environment* **466-467**, 127-135.

- Novak Babič, M., P., Z., Ženko, B., Džeroski, S., and Gunde-Cimerman, N. 2015a Yeasts and yeast-like fungi in tap water and groundwater and their transmission to household appliances. *Fungal Ecology* **10.1016/j.funeco.2015.10.001**.
- Novak Babič, M., Zalar, P., Ženko, B., Schroers, H. J., Džeroski, S., and Gunde-Cimerman, N. 2015b Candida and Fusarium species known as opportunistic human pathogens from customer-accessible parts of residential washing machines. *Fungal Biology* **119**, 95-113.
- O'Brien, D. P., Currie, B. J., and Krause, V. L. 2000 Nontuberculous mycobacterial disease in northern Australia: a case series and review of the literature. *Clinical Infectious Diseases* **31**, 958-67.
- Pedro-Botet, M. L., E., S. J., and Yu, V. L. 2002 Legionnaires' disease contracted from patient homes: the coming of the third plague? . *European Journal of Clinical Microbiology and Infectious Diseases* **21**, 699-705.
- Qu, J.-H., Yuan, H.-L., Yang, J.-S., Li, H.-F., and Chen, N. 2009 *Lacibacter cauensis* gen. nov., sp nov., a novel member of the phylum *Bacteroidetes* isolated from sediment of a eutrophic lake. *International Journal of Systematic and Evolutionary Microbiology* **59**, 1153-1157.
- Rhodes, G., Richardson, H., Hermon-Taylor, J., Weightman, A., Higham, A., and Pickup, R. 2014 Mycobacterium avium Subspecies paratuberculosis: Human Exposure through Environmental and Domestic Aerosols. *Pathogens* **3**, 577-595.
- Rimawi, B. H., Rimawi, R. H., Mirdamadi, M., Steed, L. L., Marchell, R., Sutton, D. A., Thompson, E. H., Wiederhold, N. P., Lindner, J. R., and Boger, M. S. 2013 A case of Exophiala oligosperma successfully treated with voriconazole. *Medical Mycology Case Reports* **2**, 144-147.
- Rouxel, T., Grandaubert, J., Hane, J. K., Hoede, C., van de Wouw, A. P., Couloux, A., Dominguez, V., Anthouard, V., Bally, P., Bourras, S., Cozijnsen, A. J., Ciuffetti, L. M., Degrave, A., Dilmaghani, A., Duret, L., Fudal, I., Goodwin, S. B., Gout, L., Glaser, N., Linglin, J., Kema, G. H. J., Lapalu, N., Lawrence, C. B., May, K., Meyer, M., Ollivier, B., Poulain, J., Schoch, C. L., Simon, A., Spatafora, J. W., Stachowiak, A., Turgeon, B. G., Tyler, B. M., Vincent, D., Weissenbach, J., Amselem, J., Quesneville, H., Oliver, R. P., Wincker, P., Balesdent, M.-H., and Howlett, B. J. 2011 Effector diversification within compartments of the *Leptosphaeria maculans* genome affected by Repeat-Induced Point mutations. *Nature Communications* **2**.
- Siqueira, A. F., Ormeño-Orrillo, E., Souza, R. C., Rodrigues, E. P., Almeida, L. G., Barcellos, F. G., Batista, J. S., Nakatani, A. S., Martínez-Romero, E., Vasconcelos, A. T., and Hungria, M. 2014 Comparative genomics of *Bradyrhizobium japonicum* CPAC 15 and *Bradyrhizobium diazoefficiens* CPAC 7: elite model strains for understanding symbiotic performance with soybean. *Bmc Genomics* **15**.
- Suhara, H., Maekawa, N., Kubayashi, T., Sakai, K., and Kondo, R. 2002 Identification of the basidiomycetous fungus isolated from butt rot of the Japanese cypress. *Mycoscience* **43**, 477-481.
- Takashima, M., and Nakase, T. 1999 Molecular phylogeny of the genus *Cryptococcus* and related species based on the sequences of 18S rDNA and Internal Transcribed Spacer regions. *Microbiology and Culture Collections* **15**, 35-47.
- Thomson, R., Tolson, C., Carter, R., Coulter, C., Huygens, F., and Hargreaves, M. 2013a Isolation of nontuberculous mycobacteria (NTM) from household water



- and shower aerosols in patients with pulmonary disease caused by NTM. .  
*Journal of Clinical Microbiology* **51**, 3006-3011.
- Thomson, R. M., Carter, R., Tolson, C., Coulter, C., Huygens, F., and Hargreaves, M. 2013b Factors associated with the isolation of Nontuberculous mycobacteria (NTM) from a large municipal water system in Brisbane, Australia. *BMC Microbiology* **13**, 89.
- Tortorano, A. M., Prigitano, A., Esposto, M. C., Arsic Arsenijevic, V., Kolarovic, J., Ivanovic, D., Paripovic, L., Klingspor, L., Nordøy, I., Hamal, P., Arian Akdagli, S., Ossi, C., Grancini, A., Cavanna, C., Lo Cascio, G., Scarparo, C., Candoni, A., Caira, M., Drogari Apiranthitou, M., and Group., E. W. 2014 European Confederation of Medical Mycology (ECMM) epidemiological survey on invasive infections due to *Fusarium* species in Europe. *European Journal of Clinical Microbiology and Infectious Diseases* **33**, 1623-30.
- Wiemann, P., Sieber, C. M. K., Von Bargen, K. W., Studt, L., Niehaus, E.-M., Espino, J. J., Huß, K., Michielse, C. B., Albermann, S., Wagner, D., Bergner, S. V., Connolly, L. R., Fischer, A., Reuter, G., Kleigrew, K., Bald, T., Wingfield, B. D., Ophir, R., Freeman, S., Hippler, M., Smith, K. M., Brown, D. W., Proctor, R. H., Münsterkötter, M., Freitag, M., Humpf, H.-U., Güldener, U., and Tudzynski, B. 2013 Deciphering the cryptic genome: Genome-wide analyses of the rice pathogen *Fusarium fujikuroi* reveal complex regulation of secondary metabolism and novel metabolites. *PLoS Pathogens* **9**.
- Wu, Z. H., Tsumura, Y., Blomquist, G., and Wang, X. R. 2003 18S rRNA gene variation among common airborne fungi, and development of specific oligonucleotide probes for the detection of fungal isolates. *Applied and Environmental Microbiology* **69**, 5389-5397.
- Yang, D. C., Im, W. T., Kim, M. K., Ohta, H., and Lee, S. T. 2006 *Sphingomonas soli* sp. nov., a *b*-glucosidase-producing bacterium in the family *Sphingomonadaceae* in the *a*-4 subgroup of the *Proteobacteria*. *International Journal of Systematic and Evolutionary Microbiology* **56**, 703-707.
- Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., and Glöckner, F. O. 2014 The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks. *Nucleic Acids Research* **42**, D643-D648.
- Yoon, J. H., Lee, M. H., and Oh, T. K. 2004 *Porphyrobacter donghaensis* sp. nov., isolated from sea water of the East Sea in Korea. *International Journal of Systematic and Evolutionary Microbiology* **54**, 2231-2235.
- Zeng, J. S., Sutton, D. A., Fothergill, A. W., Rinaldi, M. G., Harrak, M. J., and de Hoog, G. S. 2007 Spectrum of clinically relevant *Exophiala* species in the United States. *Journal of Clinical Microbiology* **45**, 3713-3720.